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CHEMICAL RESEARCH, DEVELOPMENT & ENGINEERING CENTER

CRDEC-CR-153

ANALYSIS OF MICROORGANISMS BY OXIDATIVE AND NON-OXIDATIVE PYROLYSIS GAS CHROMATOGRAPHY ION TRAP MASS SPECTROMETRY

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April 1992

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Aberdeen Proving Ground, Maryland 21010-5423

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1992 April	Final, 90 Jul - 91 Sep

4. TITLE AND SUBTITLE
Analysis of Microorganisms by Oxidative and Non-Oxidative Pyrolysis Gas Chromatography Ion Trap Mass Spectrometry

5. FUNDING NUMBERS
PR-1C162622A553C

6. AUTHOR(S)
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CDR, CRDEC, ATTN: SMCCR-RSL, APG, MD 21010-5423

8. PERFORMING ORGANIZATION REPORT NUMBER
CRDEC-CR-153

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
CDR, CRDEC, ATTN: SMCCR-RSL, APG, MD 21010-5423

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
COR: A. Peter Snyder, SMCCR-RSL, (410) 671-2416

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Three different bacterial species were analyzed using a quartz tube pyrolysis GC-ITD mass spectrometry system, using both oxidative and non-oxidative pyrolysis conditions. The results obtained under oxidative pyrolysis conditions were indistinguishable from those obtained with non-oxidative pyrolysis. However, differences in their lipid composition made it possible to distinguish between the three bacterial species by visual examination of appropriate data plots, using results obtained with either oxidative or non-oxidative pyrolytic conditions.

14. SUBJECT TERMS
Microorganisms, Pyrolysis, Analysis, Mass spectrometry, Bacteria, Gas chromatography, Detection

15. NUMBER OF PAGES
24

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

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PREFACE

The work described in this report was authorized under Project No. 1C162622A553C, Reconnaissance, Detection and Identification. The work was started in July 1990 and completed in September 1991.

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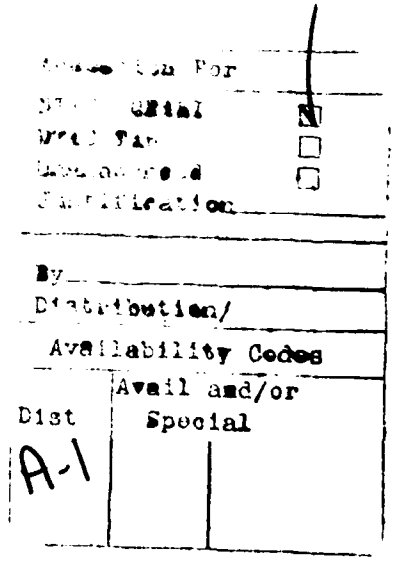
Acknowledgments

The authors would like to thank Linda G. Jarvis for the preparation and editing of this manuscript.



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ANALYSIS OF MICROORGANISMS BY OXIDATIVE AND NON-OXIDATIVE PYROLYSIS GAS CHROMATOGRAPHY ION TRAP MASS SPECTROMETRY

1. INTRODUCTION

The detection and identification of microorganisms is of significant interest in numerous scientific areas. One approach, which has found increasing utility relies on the identification of specific chemical components or biomarkers present in the microorganism of interest. Of the myriad of chemical components that can be found in microorganisms the determination of their lipid composition has been suggested to be the closest to an ideal chemotaxonomic method[1]. This is the basis of the fatty acid methyl ester (FAME) approach, which has been used successfully to characterize milligram amounts of bacteria through the saponification, derivatization and extraction of the lipid material to yield the methyl esters of the fatty acids which are then analyzed by gas chromatography[2]. The utility of this approach has been extended by the use of pyrolysis as a sample introduction technique, eliminating the need for time consuming extractions and derivatizations[3].

Pyrolysis has become an established method for introducing complex samples to a gas chromatograph (Py-GC) or a mass spectrometer (Py-MS) or a combination of the two (Py-GC-MS). However, traditionally, these techniques have yielded a wealth of non-specific low molecular weight information as the analytical interpretation of the pyrolyzate of high molecular weight biological compounds. In the last decade, however, pyrolysis has become a more effective tool in the analysis of biological material. These events largely have arisen because of the need for alternate bioanalytical determination and independent methodologies in the elucidation of biological presence with respect to convenience, experimental logistics, sensitivity, speed and sample processing and handling.

Characterization of underivatized lipid biomarker compounds with Curie-point wire Py-GC-MS was effected from whole, untreated microorganisms[3]. Significant and salient details between different and similar organisms were revealed by interpretation of the three dimensional GC-MS data space. Replacement of the Curie point micro-volume pyrolysis reactor with a commercially available and more straightforward quartz tube system resulted in essentially identical results[4]. In both these studies and in the vast majority of reports in the literature pyrolysis was effected in an inert helium atmosphere. The ability to perform the pyrolysis in air, or oxidative pyrolysis, would further minimize instrumental constraints and simplify sample handling. Consequently we are investigating the potential of oxidative pyrolysis for the identification and characterization of lipid biomarkers in microorganisms.

The attributes of oxidative pyrolysis using a quartz tube system are important because they directly impact on the 6.2 XD Chemical, Biological Mass Spectrometer (CBMS) program administered by Detection Directorate. Pyrolysis itself is an attractive sample introduction technique in terms of the generally favorable logistics and performance criteria.

Quartz tube pyrolysis offers considerable advantages over Curie point or platinum ribbon pyrolysis, both in ease of sample collection (air can be sampled and airborne particulate material collected directly into the quartz tube), sample preparation, minimal sample handling and control over the pyrolysis conditions. Unlike Curie point where the pyrolysis temperature is a discrete one, dependent upon the composition of the metal alloy used, quartz tube pyrolysis can be performed over a wide and variable temperature range, permitting the use of programmed temperature gradients. With respect to the CBMS program, oxidative pyrolysis under atmospheric air conditions eliminates the need for an external source of inert gas such as helium or nitrogen, reducing the logistics requirements.

2. EXPERIMENTAL

Staphylococcus albus, *Pseudomonas fluorescens* and *Bacillus subtilis* (BO014) were supplied by Tony P. Phillips (Chemical and Biological Defence Establishment, Porton Down, U.K.) and Leslie A. Shute (University of Bristol, Bristol, U.K.). Bacteria were grown in Lab M nutrient broth for 3 days at 37°C. The cells were harvested by centrifugation, washed and resuspended in sterile water, then killed by addition of an equal amount of 6% H_2O_2; following overnight incubation the samples were then centrifuged, washed and freeze-dried.

The Py-GC-MS equipment consisted of a Pyroprobe Model 122 power supply, a platinum coil probe, a modified pyrolysis reactor/GC interface (Chemical Data Systems, Oxford, PA), a Hewlett Packard 5890 gas chromatograph (Palo Alto, CA) and a Finnigan-MAT 700 ion trap (San Jose, CA). All pyrolyses were conducted in the pulsed mode, the final set temperature was 1000°C, and the total heating time 20s. The bacterial samples (20-50 *ug*) were sandwiched between two quartz wool plugs in the quartz tube holder and the latter was inserted into the coil of the heating probe. The pyrolysis reactor/GC inlet system were maintained at 300°C. The capillary GC column (5m x 0.32mm i.d.) (RSL-200, Alltech, Deerfield, IL) was operated with helium carrier gas at 100cm/s linear velocity. The column was temperature programmed from 100°C to 300°C at 30°C/min and then held at 300°C for 5 min. The column was connected directly into the ITD through a splitless manifold, and the 1 m transfer line portion of the capillary between the GC and the ion trap was maintained at 300°C. The teflon spacer rings in the ion trap had three 1/8" holes drilled in them to compensate for the high carrier gas flow. Spectra were accumulated over the mass range 100-650 amu at a rate of 1 scan/s for 12 min.

Under non-oxidative pyrolysis conditions, after insertion of the pyroprobe containing the sample into the pyrolysis reactor/GC interface, the entire reactor unit was flushed with a stream of helium for 60s before heating of the platinum coil. The pyrolysate was then flushed into the GC injector and onto the capillary column. Under oxidative conditions the reactor unit was opened to the atmosphere and allowed to equilibrate for 120s before the pyroprobe was inserted into the reactor unit. Immediately after insertion the platinum coil was activated, and after completion of the pyrolysis pulse, a stream of helium was directed

through the reactor unit flushing the pyrolysate into the GC injector and onto the capillary column.

3. RESULTS AND DISCUSSION

It is instructive to compare the results obtained for the analysis of a particular bacterial species using several different techniques. *Bacillus subtilis* has been analyzed by profiling of fatty acids as methyl ester derivatives using gas chromatography[5], Py-GC[6] and Py-MS[7], Py-MS/MS[8] and Py-GC-MS[3]. As might be expected, there is a trend towards greater discriminating power as the analytical technique goes from a single component system such as GC to a multicomponent Py-GC-MS or Py-MS-MS system. This is illustrated in the data analysis performed in the Py-GC-MS study[3] where several layers of information are present in the dataset. Major differences between bacterial species were readily apparent by visual inspection of the total ion current (TIC), whilst more subtle differences required analysis of the reconstructed ion chromatograms (RICs) and of individual GC-MS mass spectra.

A typical set of results that was obtained in the Curie point pyrolysis GC-ITD study[3] with *Bacillus subtilis* (BO014) is reproduced in Fig. 1. The data display procedure used in that study included examination of (a) the total ion current profile of the reconstructed chromatogram, (b) the integrated mass spectrum for the "lipid TIC region" of the chromatogram (c) selected reconstructed ion chromatograms and (d) the corresponding extracted ion mass spectra. The early portions of the TIC reproduced in Fig. 1a contained generally uninformative information. The target analyte was the underivatized lipid biomarkers that elute from the column at 5-6 min. and that portion of the chromatogram was termed the "lipid TIC region" by the authors. Two major peaks accompanied by a minor peak are clearly visible in this region of the chromatogram, although from the broad nature of the peaks and the strong likelihood of co-chromatographed compounds, it is impossible to determine the number of components present.

The integrated mass spectrum for the entire lipid region of the chromatogram is reproduced in Fig. 1b. The high molecular weight region of the mass spectrum is characterized by a series of ions differing in mass by successive 14 amu increments at m/z 494, 508, 522, 536, 550. A direct comparison of the results obtained from various bacterial phospholipids indicates that the series of ions are most likely anhydrodiacylglyceride ions, each derived from a different parent lipid compound, rather than from multiple pyrolysis and/or electron ionization induced fragmentation of a single parent compound. However, analysis is complicated since all three processes may contribute to the formation of a particular ion.

Further evidence for the diverse nature of the lipid region of the chromatogram is provided by the RICs and the accompanying extracted ion mass spectra for each of the anhydrodiacylglyceride ions in question, which are reproduced in Figs. 1c and 1d

respectively. A single fairly sharp well defined peak was observed in the RICs for each of the ions in question, and although some overlap between peaks did occur, they tended to be fairly well resolved from each other. As might be predicted, a general trend was observed in that the retention time increased with the molecular weight. Several features in the m/z 494 and 508 extracted ion mass spectra are readily apparent. The overlapping nature of some of the RICs manifests itself in the occurrence of several pseudomolecular ions in the same spectrum. For example, the extracted mass spectrum for m/z 494 also contains a prominent ion at m/z 550, whilst the extracted spectra for m/z 522 and 536 which have well resolved RICs do not contain other prominent pseudomolecular ions. The former situation thus occurs because the lower and higher molecular weight anhydrodiacylglyceride species co-elute. Certain parent ions produce characteristic mass spectral fragment ions, thus the extracted spectra of the ions at m/z 522, 536 and 550 also contain prominent fragment ions at m/z 299, 313 and 327 respectively. Although none of the extracted ion mass spectra should be considered as representing the mass spectrum of a single parent lipid compound, they do considerably simplify the situation compared to the integrated mass spectrum (Fig. 1a) obtained for the entire lipid TIC region. The extracted ion mass spectrum layer of information enables the discrimination of features not readily apparent in less sophisticated data display procedures.

The above technique of sequential examination and inquiry of a series of data display procedures of increasing specificity and sophistication and was applied to the results obtained in the study reported here. Total Ion Chromatograms (TICs) were constructed following the quartz tube Py-GC-MS of *Bacillus subtilis* under oxidative and non-oxidative pyrolysis conditions and are reproduced in Fig. 2. The initial portions of the chromatograms (0-5min) contain a series of relatively sharp peaks which consist of low to medium molecular weight (100-200 amu) compounds. These compounds are both small intact molecular species present in the bacteria, and pyrolysis induced fragments of larger components. Examination of the mass spectra of these early eluting peaks was uninformative, and provided little structural information. However, the sharp nature of the chromatographic peaks does indicate that there is an efficient transfer of the pyrolysate from the pyrolysis reactor to GC column with minimal bandbroadening and an efficient transfer from the capillary column to the mass spectrometer. Many of the early eluting peaks are of a few seconds duration, and the relatively fast mass spectrometer sampling rate of 1 scan/s results in only 2 or 3 scans per peak. As a consequence a relatively high degree of variability was observed in this region of the reconstructed ion chromatograms of bacterial analyte.

After the multiple peaks which were observed in the initial portion of the chromatogram, the detector signal level slowly returned towards the baseline. Apart from the two peaks at 6 and 7 min which represent column bleed, this region of the chromatogram contained few prominent features. In the 8-12 min region chromatograms of both the oxidative and non-oxidative pyrolysis analyses contained two prominent, broad, slightly skewed peaks. Inspection of the corresponding integrated mass spectra for this region of the chromatogram revealed a series of high molecular weight ions differing by consecutive 14 amu increments. Comparison of these spectra with reference spectra in the

literature indicated that these chromatographic peaks consisted of anhydrodiacylglycerides, and their accompanying pyrolytic and mass spectral induced fragments. These compounds are presumably produced during the pyrolysis sample processing event from the lipid cellular components of the bacteria. Both the relative (to earlier peaks in the chromatogram) and the absolute amounts of these lipid derived compounds were similar for both oxidative and non-oxidative pyrolyses, and at the TIC level of data analysis no differences between the two treatments was observed.

Integrated signal averaged mass spectra for the *Bacillus subtilis* lipid region of the chromatogram were obtained following both oxidative and non-oxidative pyrolysis and are reproduced in Fig. 3. The two mass spectra are virtually identical, both qualitatively and quantitatively, with similar absolute ion intensities recorded in both experiments. The high molecular weight region of the mass spectra contains a series of ions differing by consecutive 14 amu increments starting at m/z 452 and continuing to m/z 550, the latter of which was the most prominent ion. These ions are thought to represent dehydrated diacylglycerides which are produced as pyrolytic fragments from the higher molecular weight phospholipid components. The high molecular weight ions were accompanied by a series of mass spectral induced fragment ions also differing by successive 14 amu increments from m/z 285-341. These ions are thought to be anhydromonoacylglycerides formed by elimination of one of the acyl moieties from the anhydrodiacylglycerides. Thus the prominent fragment ion at m/z 299 can be tentatively identified as 1-pentadecyl-3-dehydroxyglycerol $[C_{14}H_{29}CO_2CH_2CH(OH)CH_2]^{+}$.

The combination of the anhydromonoacylglyceride fragment ions, which provide information on the fatty acid composition of the parent lipids, and the higher molecular weight anhydrodiacylglyceride ions which provide information about the fatty acid pairs in the parent lipid, would seem to offer a near ideal situation for the characterization of lipid composition. However, in addition to different fatty acids differing by successive methylene units, loss of successive methylene units is a characteristic mass spectral fragmentation process for this class of compound. Consequently the appearance of an ion at a certain m/z value can either signify a particular acyl substituent, or alternatively represent the elimination of a methylene subunit from a larger acyl group. The instrumentation used in this study was unable to distinguish between pyrolytic fragments and mass spectral induced fragments, however some insight into the processes involved in the formation of specific ions can be ascertained by inspection of their corresponding reconstructed ion chromatograms.

Reconstructed ion chromatograms (RICs) for the ions at m/z 494, 508, 522, 536, and 550 following oxidative and non-oxidative pyrolysis are presented in Fig. 4. With the exception of the two ions at m/z 494 and m/z 508, all of the RICs contained a single well defined peak, and as the ions increase in mass, their retention time also increases. The RICs for the ions at m/z 494 and m/z 508 contain three distinct chromatographic peaks, indicating that several isomeric forms of these ions exist, and/or a series of ions are produced as isobaric mass spectral fragments of larger compounds with different

chromatographic characteristics. The earlier low intensity eluting peaks tend to be fairly narrow, whilst the later eluting peaks are of a fairly broad nature, and coelute with higher molecular weight compounds, suggesting that they are mass spectral fragments of larger compounds. No differences could be discerned in either the intensity or the distribution between the set of RICs obtained following oxidative and non-oxidative pyrolysis.

Extracted ion mass spectra were obtained by signal averaging the eight central scans of the most intense chromatographic peak in the RICs (Fig. 4), and are reproduced in Figs. 5 following oxidative and non-oxidative pyrolysis, respectively. The extracted ion mass spectra represent the highest level (or most salient) of data processing/analysis performed in this study. Minor differences in the analytical products, between the two sample treatments, which may not be apparent in the TIC, lipid TIC mass spectra, or RICs, should be revealed following this treatment of the data. As predicted, each of the extracted ion mass spectra contained fewer ions, and were considerably simpler than the lipid TIC mass spectrum. With the exception of the extracted ion mass spectra for m/z 494 and 508, the chosen extracted ion was the most prominent parent ion in each of the mass spectra, and minimal overlap was apparent from other high molecular weight ions. The information in the extracted ion mass spectra for the m/z 494 and 508 ions was considerably more complicated than in the other mass spectra. Examination of the RICs for these two ions had suggested that they were mass spectral fragment ions, rather than pyrolysis products as was the case for the other ions studied, and inspection of the extracted ion mass spectra tends to confirm this.

For the other ions studied, it was possible to discern a temporal relationship between certain mass spectral fragment ions and higher molecular weight pseudomolecular ions. For example the fragment ion at m/z 313 was almost exclusively associated with the ion at m/z 536. Similarly the fragment at m/z 327 was only present in the spectra which contained a prominent m/z 550 ion. A prominent ion at m/z 299 was present in all of the extracted ion mass spectra, indicating that this may be a common fragment formed from a variety of different parent ions. No significant differences were observed in any of the extracted ion mass spectra between oxidative and non-oxidative pyrolysis.

An identical data processing and data analysis technique was applied to the results obtained with the other two bacterial species, namely *Staphylococcus albus* and *Pseudomonas fluorescens*, and the results obtained after oxidative and non-oxidative pyrolysis are presented in Figs. 6-9. No differences were observed in any of the levels of data analysis between the two sample treatments. However, differences are readily apparent between the results obtained for the three bacterial species investigated.

The early regions of the TICs for *Staphylococcus albus* and *Pseudomonas fluorescens* are similar to those obtained with *Bacillus subtilis*, however, differences are observed in the lipid TIC region of each organism's chromatogram. Figures 6 and 7 represent the oxidative and non-oxidative pyrolysis Py-GC-MS data, respectively, of *Staphylcoccus albus* and Figs. 8 and 9 represent that of *Pseudomonas fluorescens*, respectively. This region of the TIC for

B. subtilis (Fig. 2) contained two prominent peaks, whilst that for *S. albus* (Figs. 6A, 7A) contains a single broad tailing peak. The corresponding lipid region of *P. fluorescens* (Figs. 8A, 9A) contained a broad peak, and in all cases virtually identical results were obtained after oxidative and non-oxidative pyrolysis. One could easily discriminate between *B. subtilis* and the other two bacteria by visual inspection of the lipid region of the reconstructed TIC, whilst only slight differences of the lipid TIC made the discrimination of *S. albus* and *P. fluorescens* difficult.

The similarities in the lipid TICs between *S. albus* and *P. fluorescens* were also apparent by comparison of their respective total lipid mass spectra (frame B in Figs. 6-9). The ion at m/z 522-523 was the most prominent high molecular weight ion in both spectra (compared to m/z 550 for *B. subtilis*, Fig. 3). It was, however, considerably more intense in the spectra of *S. albus* when compared to the spectra of *P. fluorescens*. This was readily apparent by comparison of the ratios of the intensities of m/z 523/536. No significant differences could be observed between the lipid TIC mass spectra obtained from either oxidative or non-oxidative pyrolysis, and similarly no differences were found in either the RICs or the extracted ion mass spectra between oxidative pyrolysis and non-oxidative pyrolysis for any of the bacteria investigated.

The RICs (Frame C in Figs. 6-9) for *S. albus* and *P. fluorescens* were very similar to each other, and similar to those for *B. subtilis (Fig. 4)*. Differences were apparent in the RICs for m/z 494 and 508 for *S. albus* and *P. fluorescens* with respect to *B. subtilis*. They both displayed a small early eluting peak followed by a large broad peak coeluting with higher molecular weight compounds, in contrast to the three distinct peaks observed with *B. subtilis*. Comparison of the intensity ratio of the ions at m/z 522/536 indicated a value of 3.0 for *B. subtilis* and *P. fluorescens* and a 5.0 ratio for *S. albus* and illustrates the predominance of the m/z 522 ion in the mass spectra of *S. albus*. Inspection of the extracted ion mass spectra (frame D of Figs. 6-9 and Fig. 5) highlighted only slight differences. There were many similarities between the extracted ion mass spectra for the three bacteria. The extracted ion mass spectra for m/z 522 were virtually identical for all three bacteria. The extracted ion mass spectra for m/z 536 were identical for *S. albus* and *B. subtilis* whilst the spectra for *P. fluorescens* contained a small m/z 536 ion and a prominent m/z 522 ion. The extracted mass spectra for m/z 550 were fairly similar for *P. fluorescens* and *S. albus*. In the equivalent mass spectrum, *B. subtilis* contained a much more prominent m/z 327 fragment ion, whilst the m/z 550 extracted ion mass spectra for *S. albus* and *P. fluorescens* again had an intense m/z 522 ion. In an analogous fashion the extracted ion mass spectra for m/z 494 and 508 for both *S. albus* and *P. fluorescens* were dominated by the m/z 522-523 ion pair (which was the most prominent high mass ion in the lipid TIC mass spectra) whilst the corresponding spectra for *B. subtilis* were dominated by the m/z 550 ion and accompanying m/z 327 fragment ion.

The extracted ion mass spectra can be considered to be sets of spectra taken at particular times throughout the lipid region of the chromatogram, and as such contain subsets or portions of information from the lipid TIC mass spectrum. This is because the

lipid TIC mass spectrum is an averaged composite of the extracted ion mass spectra for all the lipid components. Slight differences in the lipid composition are much more readily apparent from examination of the separate extracted ion mass spectra than from examination of the entire lipid TIC mass spectrum.

A comprehensive comparison of quartz tube pyrolysis and Curie point pyrolysis is premature and outside the scope of this report. It is, however, instructive to compare the results obtained in the present study using quartz tube Py-GC-MS under oxidative and non-oxidative conditions with those reported using a Curie point Py-GC-MS system[3] for *Bacillus subtilis*. Both the lipid TIC mass spectra, and the extracted ion mass spectra were essentially identical following either Curie point or quartz tube pyrolysis. Components in the lipid region of the total ion chromatogram were better resolved in the Curie point pyrolysis study than in the quartz tube study, and to some extent this was also apparent in the RICs for the various ions.

4. CONCLUSIONS

Despite fairly extensive data analysis, there was no discernable difference in the analytical information following either oxidative or non-oxidative pyrolysis. This finding was somewhat surprising, since one might expect pyrolysis at 1000°C in an oxygen containing atmosphere to result in the extensive combustion of a sample of biological origin. However, there was no increase in the relative amounts of non-discriminative low molecular weight compounds which would indicate extensive combustion/fragmentation, and the ion intensities of the high molecular weight lipid compounds were virtually identical for both treatments. Pulsed pyrolysis which was used in these experiments as opposed to slow linear temperature programmed pyrolysis, produces a very fast heating of the sample. This results in a subsequent rapid pyrolyzate removal from the heating zone and could account for the mininmal formation of combustion products.

For the laboratory instrumentation used in this study, the ability to perform pyrolysis without the need for a bottled supply of inert gas is of little logistical value. In fact, because the capillary GC column requires either helium or hydrogen as a carrier gas, it is more practical to use an inert gas. Exposure of the GC column to oxygen at an elevated temperature strips the chemically bonded stationary phase from the column, a process termed column bleed. Column bleed results in elevated background detector levels, spurious GC peaks and drastically reduces column life. It is envisioned that the CBMS unit will utilize oxidative pyrolysis as a sample processing/introduction technique, and in order for this instrumentation to be effective, it will require the collection of background and reference data. Our findings indicate that the large amount of published reference data already obtained under non-oxidative pyrolysis conditions could very well be applicable to an oxidative pyrolysis system. Furthermore additional reference data can easily be obtained using currently available commercial instrumentation.

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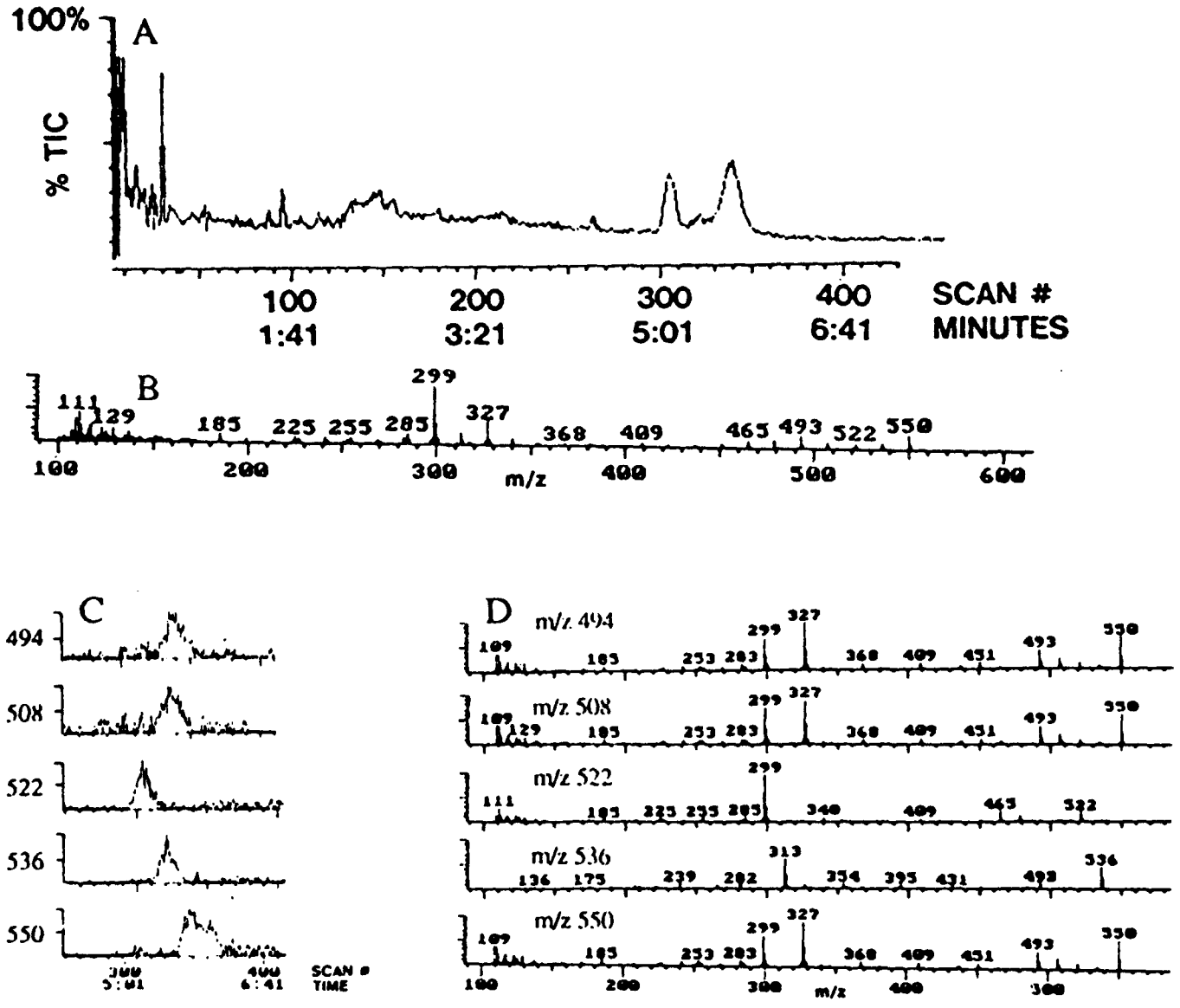

Figure 1. Curie point pyrolysis GC-ITD data for *Bacillus subtilis*. Total Ion Chromatogram (A), Total Lipid Mass Spectrum (B), Reconstructed Ion Chromatograms (C), Extracted Ion Mass Spectra (D).

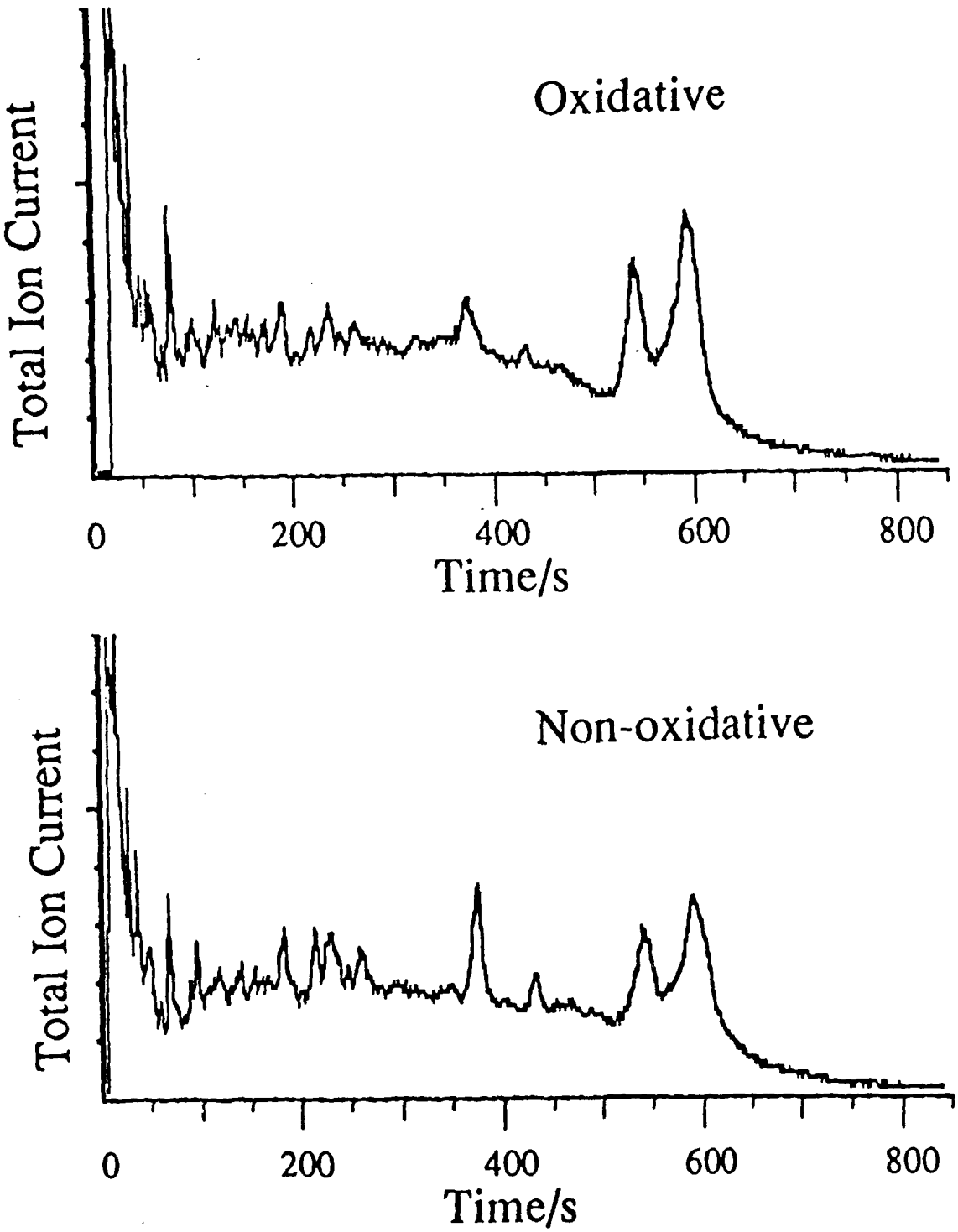

Figure 2. Total Ion Chromatograms for *Bacillus subtilis* under oxidative and non-oxidative pyrolysis conditions

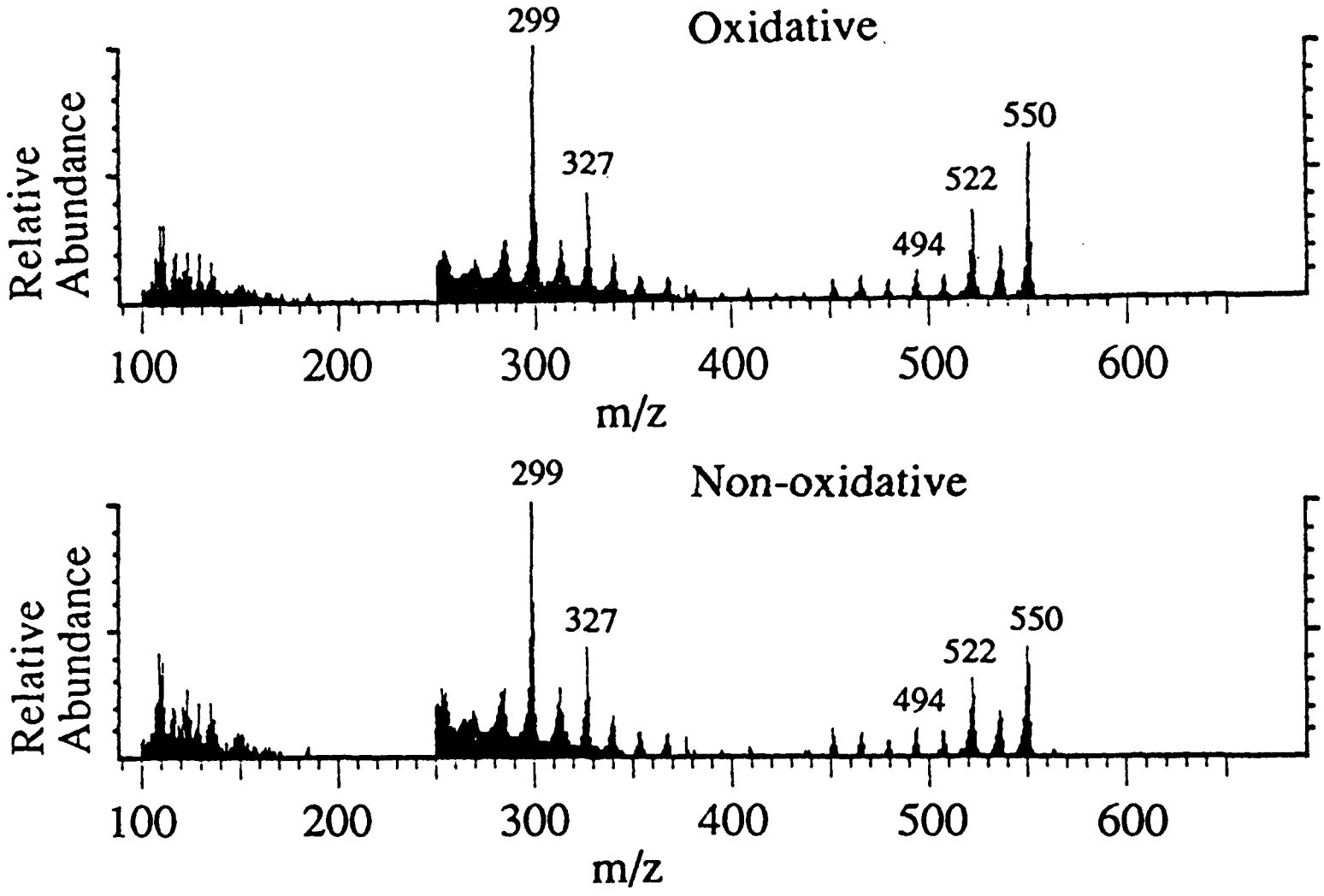

Figure 3. Total Lipid Mass Spectra for *Bacillus subtilis* under oxidative and non-oxidative pyrolysis conditions

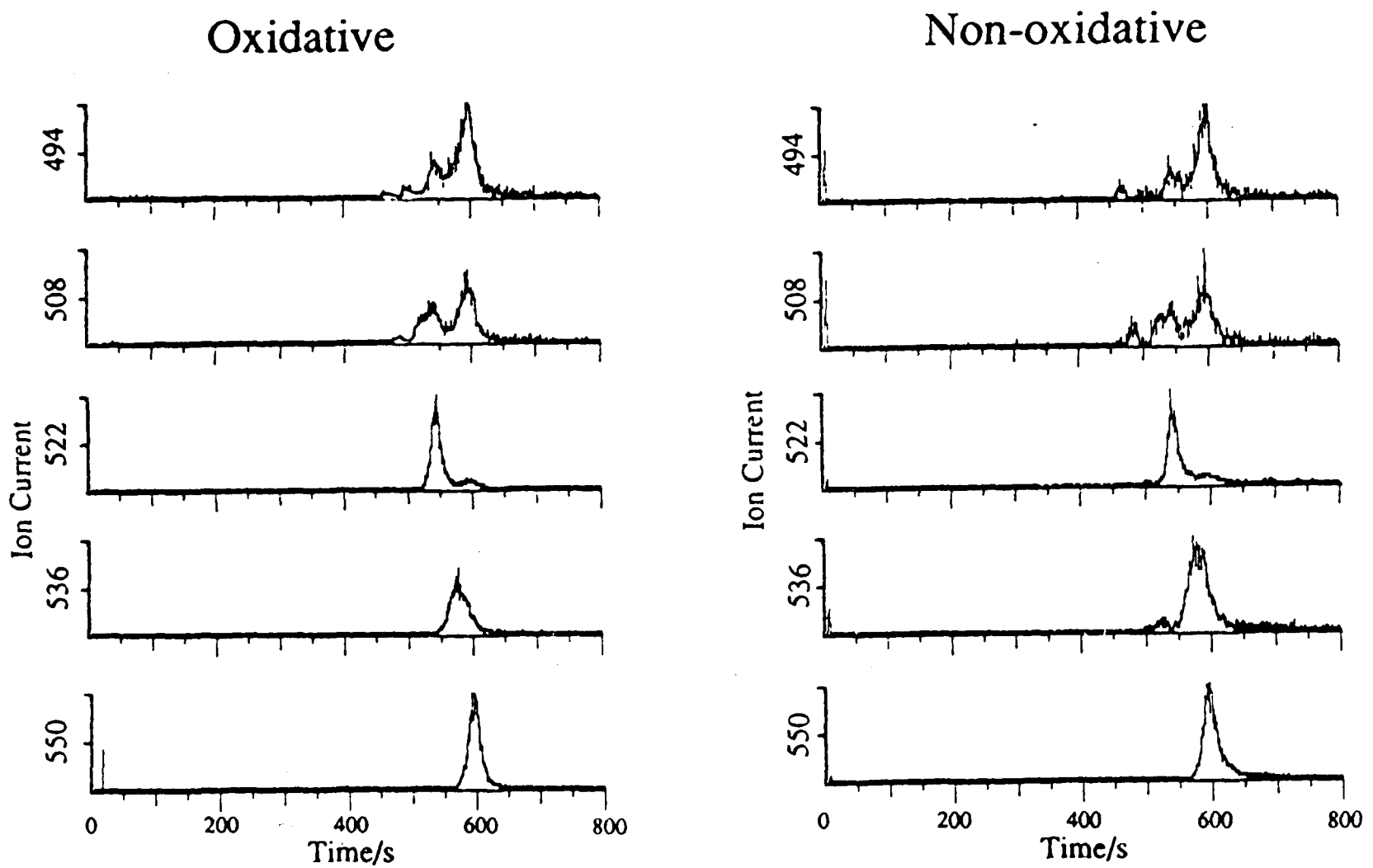

Figure 4. Reconstructed Ion Chromatograms for *Bacillus subtilis* under oxidative and non-oxidative pyrolysis conditions

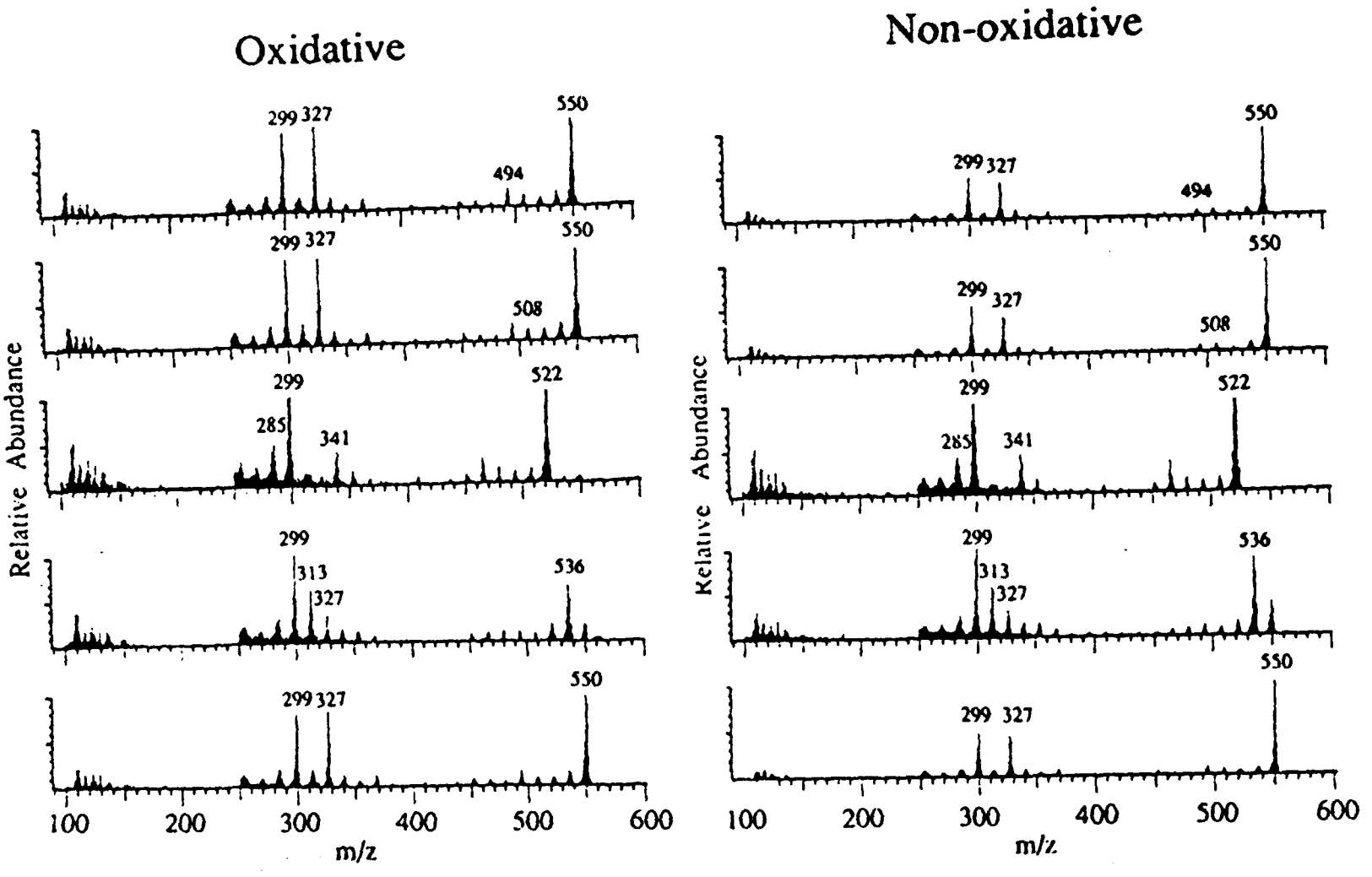

Figure 5. Extracted Ion Mass Spectra for *Bacillus subtilis* under oxidative and non-oxidative pyrolysis conditions

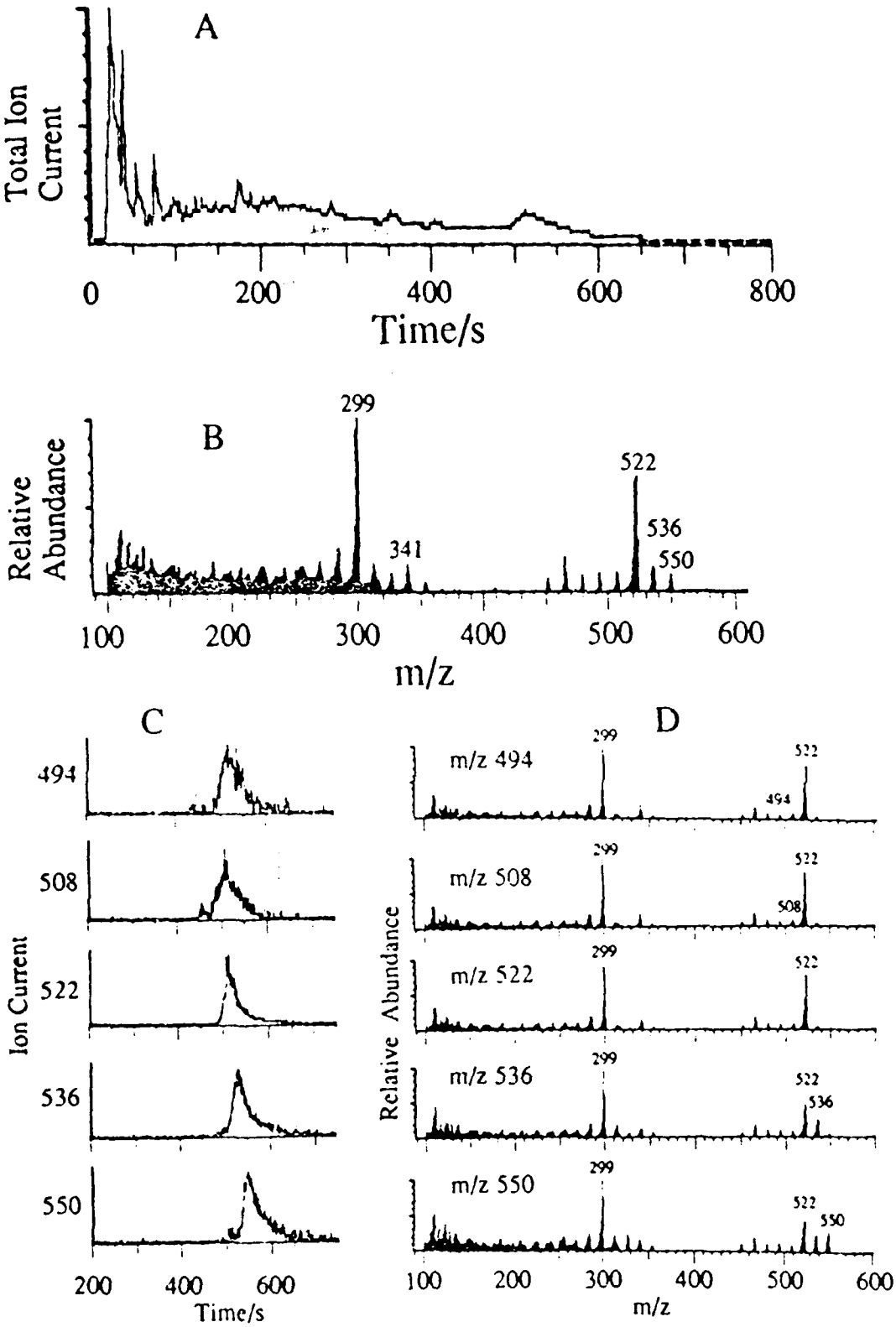

Figure 6. Total Ion Chromatogram (A), Total Lipid Mass Spectra (B), Reconstructed Ion Chromatograms (C), and Extracted Ion Mass Spectra (D) for *Staphylococcus albus* under oxidative pyrolysis.

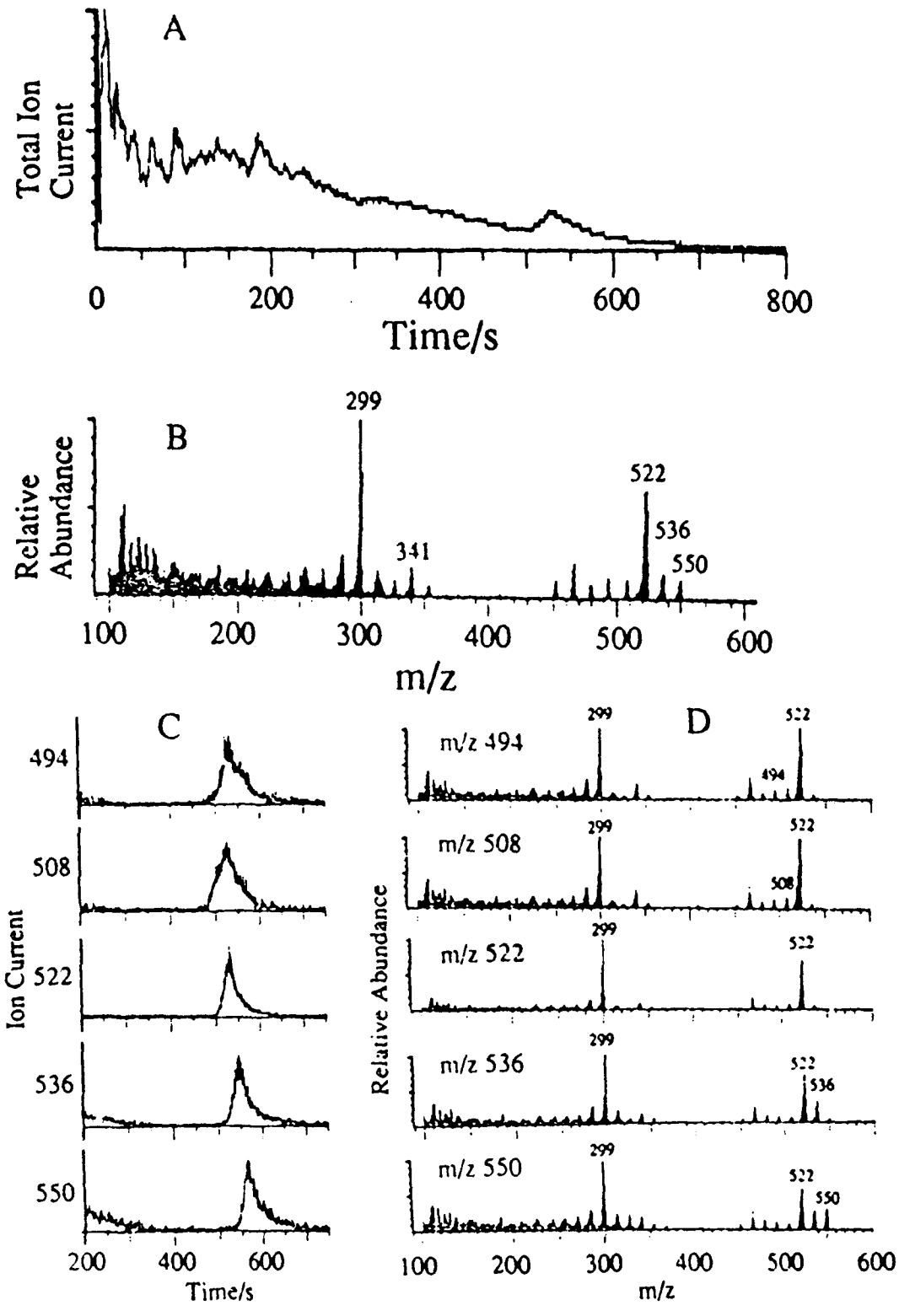

Figure 7. Total Ion Chromatogram (A), Total Lipid Mass Spectra (B), Reconstructed Ion Chromatograms (C), and Extracted Ion Mass Spectra (D) for *Staphylococcus albus* under non-oxidative pyrolysis.

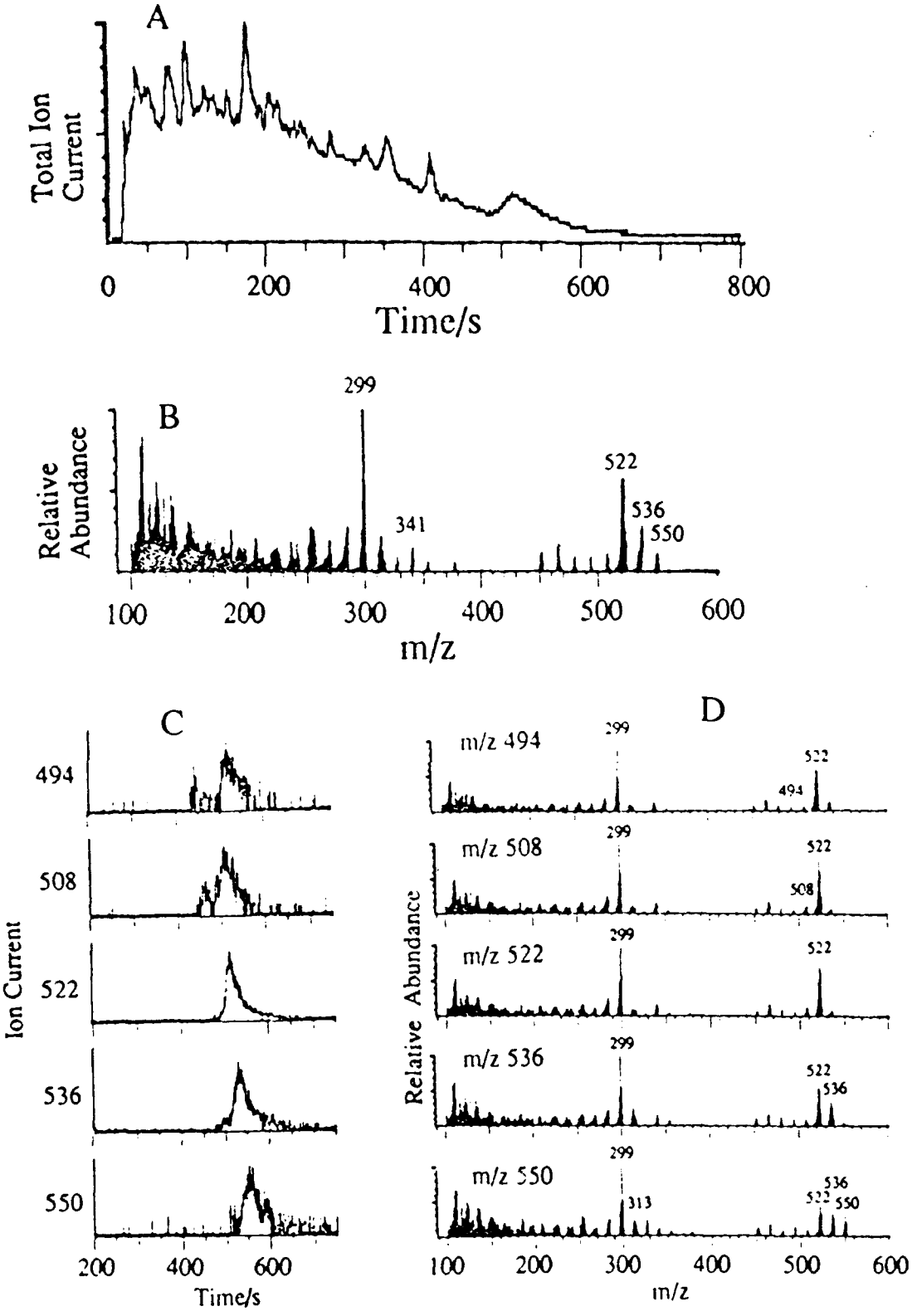

Figure 8. Total Ion Chromatogram (A), Total Lipid Mass Spectra (B), Reconstructed Ion Chromatograms (C), and Extracted Ion Mass Spectra (D) for *Pseudomonas fluorescens* under oxidative pyrolysis.

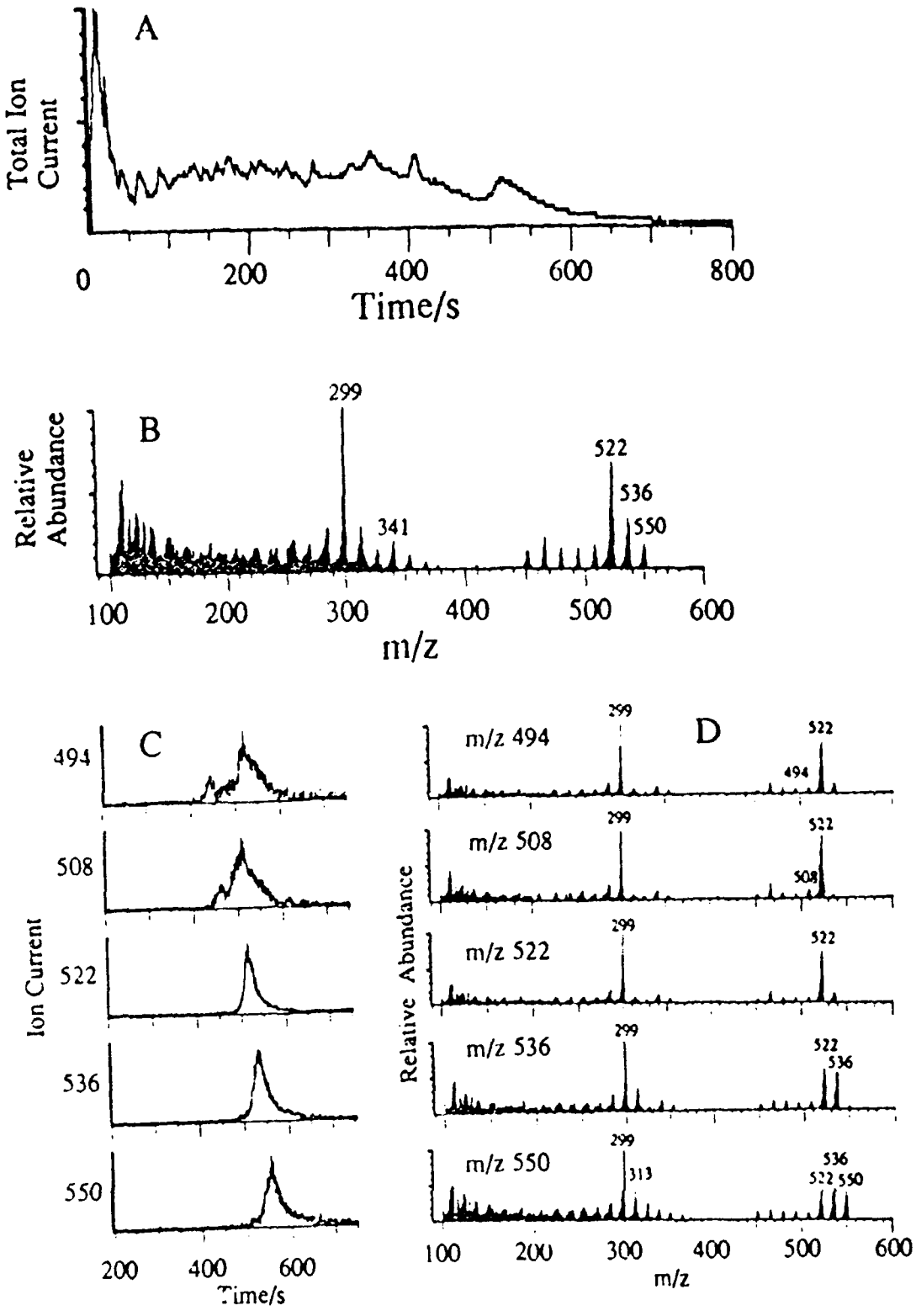

Figure 9. Total Ion Chromatogram (A), Total Lipid Mass Spectra (B), Reconstructed Ion Chromatograms (C), and Extracted Ion Mass Spectra (D) for *Pseudomonas fluorescens* under non-oxidative pyrolysis.